JOURNAL OF CLINICAL MICROBIOLOGY, July 2007, p. 2105–2109 Vol. 45, No. 7
0095-1137/07/$08.00+0 doi:10.1128/JCM.00210-07
Copyright © 2007, American Society for Microbiology. All Rights Reserved.

Simultaneous Detection and High-Throughput Identification of a Panel of RNA Viruses Causing Respiratory Tract Infections▽

Haijing Li,[1] Melinda A. McCormac,[2] R. Wray Estes,[2] Susan E. Sefers,[2] Ryan K. Dare,[5] James D. Chappell,[2,3] Dean D. Erdman,[5] Peter F. Wright,[2,3,4] and Yi-Wei Tang[1,2]*

Departments of Medicine,[1] Pathology,[2] Pediatrics,[3] and Microbiology and Immunology,[4] Vanderbilt University School of Medicine, Nashville, Tennessee 37232, and Respiratory and Gastroenteritis Viruses Branch, Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333[5]

Received 26 January 2007/Returned for modification 19 March 2007/Accepted 9 May 2007

Clinical presentations for viral respiratory tract infections are often nonspecific, and a rapid, high-throughput laboratory technique that can detect a panel of common viral pathogens is clinically desirable. We evaluated two multiplex reverse transcription-PCR (RT-PCR) products coupled with microarray-based systems for simultaneous detection of common respiratory tract viral pathogens. The NGEN respiratory virus analyte-specific assay (Nanogen, San Diego, CA) detects influenza A virus (Flu-A) and Flu-B, parainfluenza virus 1 (PIV-1), PIV-2, and PIV-3, and respiratory syncytial virus (RSV), while the ResPlex II assay (Genaco Biomedical Products, Inc., Huntsville, AL) detects Flu-A, Flu-B, PIV-1, PIV-2, PIV-3, PIV-4, RSV, human metapneumovirus (hMPV), rhinoviruses (RhVs), enteroviruses (EnVs), and severe acute respiratory syndrome (SARS) coronavirus (CoV). A total of 360 frozen respiratory specimens collected for a full year were tested, and results were compared to those obtained with a combined reference standard of cell culture and monoplex real-time TaqMan RT-PCR assays. NGEN and ResPlex II gave comparable sensitivities for Flu-A (82.8 to 86.2%), Flu-B (90.0 to 100.0%), PIV-1 (87.5 to 93.8%), PIV-3 (66.7 to 72.2%), and RSV (63.3 to 73.3%); both assays achieved excellent specificities (99.1 to 100.0%) for these five common viruses. The ResPlex II assay detected hMPV in 13 (3.6%) specimens, with a sensitivity of 80.0% and specificity of 99.7%. The ResPlex II assay also differentiated RSV-A and RSV-B and gave positive results for RhV and EnV in 31 (8.6%) and 19 (5.3%) specimens, respectively. PIV-2, PIV-4, and SARS CoV were not detected in the specimens tested. The two systems can process 80 (NGEN) and 96 (ResPlex II) tests per run, with a hands-on time of approximately 60 min and test turnaround times of 6 h (ResPlex II) and 9 h (NGEN). Multiple-panel testing detected an additional unsuspected 9 (3.4%) PIV-1 and 10 (3.7%) PIV-3 infections. While test sensitivities for RSV and PIV-3 need improvement, both the NGEN and ResPlex II assays provide user-friendly and high-throughput tools for simultaneous detection and identification of a panel of common respiratory viral pathogens in a single test format. The multiplex approach enhances diagnosis through detection of respiratory viral etiologic agents in cases in which the presence of the agent was not suspected and a test was not ordered by the clinicians.

Acute respiratory virus infections are among the most common causes of human disease. Infants, the elderly, and individuals with compromised cardiac, pulmonary, or immune systems are at greatest risk of serious complications from these viruses. Well-recognized respiratory viral pathogens include influenza virus A (Flu-A) and Flu-B, parainfluenza virus 1 (PIV-1), PIV-2, PIV-3 and PIV-4, human metapneumovirus (hMPV), respiratory syncytial virus (RSV), rhinovirus (RhV), and enterovirus (EnV), which can cause a spectrum of illnesses such as upper and lower respiratory infections, otitis media, parotitis, and encephalitis. Respiratory infections caused by these viruses usually present with similar signs and symptoms that are nearly indistinguishable by clinical diagnosis. Few influenza virus infections in children are recognized by the treating clinician in the inpatient or outpatient setting (33). Rapid detection of these pathogens is very important for initiating antiviral therapy, avoiding unnecessary antimicrobial therapy, preventing nosocomial spread, decreasing the duration of hospital stays, and reducing management costs (17, 41).

Traditionally, laboratory diagnostic testing for respiratory viruses has been limited to choosing between viral antigen tests, which often lack sensitivity and specificity, and cell culture-based tests, which generally have longer turnaround time (TAT) for diagnosis and treatment of acute illness. Molecular assays have the potential for high sensitivity, with assay TAT on the order of a few hours and foreseeable compatibility with high-throughput batch processes. The superiority of PCR and reverse transcription-PCR (RT-PCR) assays over conventional methods for the diagnosis of respiratory viral infections has been established previously (3, 37, 40). However, these organism-specific RT-PCR assays, which require separate amplification of each virus of interest, are resource intensive; when a respiratory viral infection is considered, the list of possible etiologies is necessarily long, since there is substantial overlap in clinical presentations.

Respiratory screen direct immunofluorescence staining, which is a multiplex assay with sensitivity comparable to that of culture, has been used in the clinical setting to screen and detect a panel of common respiratory viral pathogens (26).

* Corresponding author. Mailing address: Molecular Infectious Diseases Laboratory, Vanderbilt University Hospital, 4605 TVC, Nashville, TN 37232-5310. Phone: (615) 322-2035. Fax: (615) 343-8420. E-mail: yiwei.tang@vanderbilt.edu.

▽ Published ahead of print on 16 May 2007.

Multiplex RT-PCR-based techniques have been widely reported for simultaneous detection of a panel of respiratory viral pathogens in a single reaction (4, 6, 9, 11, 12, 14, 15, 18, 23, 27, 32, 34, 36, 38). Although a monoplex assay can be readily optimized for a particular target, a combination of reagents to detect multiple pathogens in a single reaction generally results in loss of sensitivity for each of the individual target species. It is difficult to optimize the conventional multiplex PCR procedure, because each amplification target corresponds to a set of primers with a unique set of optimal annealing conditions. Multiple primers included in one tube also may result in primer-primer interference and nonspecific nucleic acid amplification. For fluorescence-reporting real-time assays, physical limits are imposed by the requirement for nonoverlapping spectral ranges for each reporting dye; for most instruments, this limits a multiplex assay to a maximum of five targets (31).

Novel multiplex RT-PCR-based systems for detection of a panel of respiratory viruses have been previously described (7, 25). Some of these are commercial products, including the Seeplex RV detection kit from Seegene Inc. (Seoul, Korea), the MultiCode-PLx respiratory virus panel from EraGen Biosciences (Madison, WI), and the ID-Tag respiratory viral panel from Tm Bioscience Corp. (Toronto, Canada). In this study, we evaluated two new products, the NGEN respiratory virus (RVA) analyte-specific reagent (ASR) assay from Nanogen (San Diego, CA) and the ResPlex II assay from Genaco Biomedical Products, Inc. (Huntsville, AL), using a panel of routinely collected respiratory specimens submitted for diagnostic testing. The NGEN RVA ASR assay, which combines multiplex amplification (11) with an electronic microarray that utilizes fluorescently labeled probes, detects six viruses: Flu-A and Flu-B, PIV-1, PIV-2, and PIV-3, and RSV. The ResPlex II assay, which uses a target-enriched multiplex RT-PCR amplification technique followed by Luminex liquid chip hybridization and identification (16), detects 12 viruses: Flu-A and Flu-B, PIV-1, PIV-2, PIV-3, and PIV-4, RSV-A and RSV-B, hMPV, RhV, EnV, and severe acute respiratory syndrome (SARS) coronavirus (CoV). Both systems incorporate multiplex RT-PCR and fluorescent probe detection microarrays, providing simultaneous detection and rapid high-throughput identification of a panel of common viruses causing respiratory tract infections.

(This study was presented in part at the Association for Molecular Pathology Annual Meeting, Orlando, FL, 16 to 19 November, 2006, and at the Annual Meeting of the Pan American Society for Clinical Virology, Clearwater Beach, FL, 29 April to 2 May, 2007.)

MATERIALS AND METHODS

Clinical samples. Nasal wash (NW) and nasopharyngeal swab (NPS) specimens were collected over the study period from February 2005 to February 2006. The specimens were submitted to the Clinical Virology Laboratory at Vanderbilt University Medical Center for detection of one or more respiratory viruses by viral antigen testing or viral culture. For each month, all specimens with positive antigen and/or culture results were included in the analysis, and consecutive specimens with negative results from the beginning of the month were added to achieve a total of 30 specimens. The number of antigen- and/or culture-positive specimens analyzed for any given virus was capped at 10 within a single month. Specimens with sufficient residual volume after routine diagnostic testing were stored at −80°C for further study.

Total RNA extraction. A QIAamp MinElute viral vacuum kit (QIAGEN Inc., Valencia, CA) was used to extract total nucleic acid from the frozen NW or NPS specimens as previously described (35). Briefly, 0.9 ml of lysis buffer was added to 0.2 ml of a thawed NW or NPS specimen and mixed with 75 μl of protease followed by addition of 550 μl of buffer AL-carrier RNA. After incubation at 56°C for 15 min, 600 μl of ethanol was added, and the mixture was incubated for 5 additional minutes. The entire volume of sample was added to a spin column connected to a vacuum manifold. The sample was drawn through the column, allowing nucleic acids to bind the silica membrane. Several washes were performed, and total nucleic acid was eluted in 55 μl of RNase-free water.

Nanogen NGEN RVA ASR assays. The reagents were used in two steps: (i) simultaneous amplification of Flu-A, Flu-B, PIV-1, PIV-2, PIV-3, and RSV in a multiplex format using RT-PCR (11) and (ii) detection and identification of pathogen sequences by use of a NanoChip 400 electronic microarray (22). For reverse transcription, 3 μl of eluted RNA was added to 17 μl of RT mixture. For the PCR step, 10 μl of the RT reaction mixture was combined with 40 μl of the PCR mixture. Following amplification, 9 μl of amplified product was diluted in 66 μl of Cap Down sample buffer A and placed on the NanoChip instrument. Viral RNA-positive controls (Prodesse Inc., Madison, WI) and negative controls were used in each run. Data were analyzed by using a protocol generated from the NanoChip 400 protocol template library (version 1.00).

Genaco ResPlex II assay. The ResPlex II assay system entails the simultaneous amplification of Flu-A, Flu-B, PIV-1, PIV-2, PIV-3, PIV-4, RSV-A, RSV-B, hMPV, RhV, EnV, and SARS CoV in a single reaction using TemPlex technology (16) followed by product detection and identification using a Luminex suspension microarray (8). In brief, 6 μl ResPlex II SuperPrimers were added to 25 μl of QIAGEN HotStarTaq master mix (QIAGEN Inc, Valencia, CA) followed by addition of 5 μl extracted nucleic acid and 14 μl water for a final volume of 50 μl. Amplification was initiated with reverse transcription at 50°C for 30 min followed by use of the five-stage Templex cycling program as previously described (8, 16). Amplified products were identified using a suspension array for multiplex detection and a Luminex 100 instrument (Luminex, Austin, TX) as previously described (8, 16). Results for each channel are expressed as the median fluorescence intensity (MFI) value. The cutoff value for each target was determined as the sum of the mean plus four times the standard deviations of the negative controls.

Real-time TaqMan RT-PCR assays. Seven real-time RT-PCR assays that detect Flu-A, Flu-B, PIV-1, PIV-2, PIV-3, RSV, and hMPV were performed using an ABI PRISM 7700 sequence detection system (Applied Biosystems, Foster City, CA) as previously described (10, 35). In brief, 25 μl reaction mixture containing 5 μl extracted RNA, 0.5 μM each primer, and 0.2 μM TaqMan probe was mixed with 25 μl TaqMan One-Step RT-PCR 2× Master Mix (Applied Biosystems). Reaction conditions were designed as follows: RT at 48°C for 30 min, initial denaturation at 95°C for 10 min, and 40 cycles of denaturation (95°C for 15 s) and annealing and extension (60°C for 1 min) (10, 35). Primers and probe sets for these seven viruses were modified from those either developed at the Centers for Disease Control and Prevention (available upon request) or previously published (5, 19). Probes were dual labeled with the reporter dye 6-carboxy fluorescein at the 5′ end and either 6-carboxytetramethyrhodamine or a minor groove binder quencher at the 3′ end.

Viral culture. Culturing was performed on a subset of the specimens as ordered by the treating physicians. Primary rhesus monkey kidney cells and human epidermoid carcinoma (HEp-2) cells (Viromed Laboratories, Minnetonka, MN, and Diagnostic Hybrids, Athens, OH) were used for isolation of Flu-A, Flu-B, PIV-1, PIV-2, PIV-3, and RSV by standard methods. Fresh NW or NPS specimens were inoculated onto subconfluent monolayers of primary rhesus monkey kidney and HEp-2 cells and monitored for the appearance of characteristic cytopathic effects or development of hemadsorbing capacity by use of guinea pig erythrocytes. Culture confirmation was performed by immunofluorescence microscopy using type-specific monoclonal antibodies according to the instructions of the manufacturer (Chemicon International, Temecula, CA).

Evaluation references. All positive cell culture and/or real-time RT-PCR results were considered true positives for Flu-A, Flu-B, PIV-1, PIV-2, PIV-3, RSV, and hMPV. The antigen test results were not included for validation purposes due to their relative low sensitivities and specificities. The Genaco ResPlex II results obtained with other viruses were recorded but not validated.

RESULTS

A total of 360 clinical specimens, 135 (37.5%) NW and 225 (62.5%) NPS, were tested. Among patients from whom these specimens were collected, 195 (54.2%) were male and 283

TABLE 1. Sensitivity and specificity of Nanogen GNEN and Genaco ResPlex II for detecting respiratory viruses from 360 clinical specimens[a]

Virus[b]	NGEN						ResPlex II					
	No. of specimens:				Sensitivity (%)	Specificity (%)	No. of specimens:				Sensitivity (%)	Specificity (%)
	$S^+ T^+$	$S^- T^+$	$S^+ T^-$	$S^- T^-$			$S^+ T^+$	$S^- T^+$	$S^+ T^-$	$S^- T^-$		
Flu-A	25	0	4	331	86.2	100.0	24	0	5	331	82.8	100.0
Flu-B	10	2	0	348	100.0	99.4	9	0	1	350	90.0	100.0
PIV-1	15	3	1	341	93.8	99.1	14	0	2	344	87.5	100.0
PIV-3	12	0	6	342	66.7	100.0	13	0	5	342	72.2	100.0
RSV	19	2	11	328	63.3	99.4	22	0	8	330	73.3	100.0
hMPV	NA	NA	NA	NA	NA	NA	12	1	3	344	80.0	99.7

[a] No PIV-2 was detected. S, reference standard; T, NGEN or ResPlex II test; NA, not applicable.
[b] One coinfection with Flu-B and hMPV was detected. ResPlex II detected an additional 13 (3.6%) enterovirus infections, 25 (6.9%) rhinovirus infections, and 6 (1.7%) enterovirus-rhinovirus coinfections. ResPlex II results for enteroviruses and rhinoviruses were not validated.

(78.6%) were ≤18 years old. The mean and median ages of the study population were 10.8 and 1.3 years, respectively. Comprehensive viral culturing of Flu-A, Flu-B, PIV-1, PIV-2, PIV-3, and RSV was performed for 93 (25.8%) of the study specimens. Culturing for RSV only was performed for an additional 44 (12.2%) specimens. Culture results were positive for 71 specimens, including 20 (28.2%) Flu-A, 8 (11.3%) Flu-B, 9 (12.7%) PIV-1, 9 (12.7%) PIV-3, and 25 (35.2%) RSV. Monoplex RT-PCR detected at least one viral pathogen in 110 specimens, including 29 (26.4%) Flu-A, 8 (7.3%) Flu-B, 14 (12.7%) hMPV, 13 (11.8%) PIV-1, 16 (14.5%) PIV-3, 29 (26.4%) RSV, and 1 (0.9%) Flu-B and hMPV.

We first assessed the performance of the NGEN and ResPlex II assays using a standard reference of combined results from viral culture and the monoplex RT-PCR assay (Table 1). PIV-2 was not detected by culture or molecular methods. NGEN detected 25 (6.9%) Flu-A specimens, 12 (3.3%) Flu-B, 18 (5.0%) PIV-1, 12 (3.3%) PIV-3, and 21 (5.8%) RSV, corresponding to sensitivities of 86.2%, 100.0%, 100.0%, 93.8%, and 63.3% and specificities of 100.0%, 99.4%, 99.1%, 100.0%, and 99.4%, respectively. A total of seven false-positive results were observed in the NGEN asssay, including two Flu-B, three PIV-1, and two RSV. ResPlex II performed comparably, demonstrating sensitivity of 82.8% for Flu-A, 90.0% for Flu-B, 87.5% for PIV-1, 72.2% for PIV-3, 73.3% for RSV, and 80.0% for hMPV, with 100.0% specificity for Flu-A, Flu-B, PIV-1, PIV-3, and RSV and 99.7% specificity for hMPV. ResPlex II also differentiated RSV-A and RSV-B and gave 31 (8.6%) RhV- and 19 (5.3%) EnV-positive results.

Cross-reactivity between PIV-1 and PIV-3 was observed in 8 (29.6%) of 27 specimens positive for PIV-1 or PIV-3 by ResPlex II. A true positive result was judged according to the higher MFI value, which was ≥2× the lower value (data not shown). This cross-reactivity was not observed with the NGEN assay. Neither PIV-4 nor SARS CoV was detected using ResPlex II.

The performance parameters of NGEN and ResPlex II for simultaneous detection of a panel of common respiratory viral pathogens were assessed and compared (Table 2). NGEN detected six viruses in one reaction with a maximum processing capacity of 80 specimens and controls per run. In comparison, ResPlex II detected 12 viruses in one reaction with a maximum processing capacity of 96 specimens and controls per run. The hands-on time and test TAT for a full run were 70 min and 9 h for NGEN and 55 min and 6 h for ResPlex II, respectively. Both the NGEN and ResPlex II assays afforded high throughput and required significantly lesser specimen volumes than the monoplex-formatted real-time TaqMan RT-PCR assays. However, unlike real-time assays, both multiplex assays require postamplification PCR product manipulation that could lead to laboratory contamination with amplified DNA.

We examined individual virus detection rates with respect to suspected agents considered by the ordering physician. As shown in Table 3, clinicians were highly cognizant of infections by Flu-A, Flu-B, and RSV, with only three specimens in which viruses were detected but not sought by the clinician. In contrast, 9 PIV-1 (3.4%)- and 10 PIV-3 (3.7%)-positive results

TABLE 2. Comparison of technical parameters among Nanogen GNEN, Genaco ResPlex II, and monoplex TaqMan methods for detection of RNA virus respiratory pathogens

Parameter	GNEN	ResPlex II	TaqMan
Amplification platform	Multiplex RT-PCR	Multiplex RT-PCR	Monoplex RT-PCR
Detection format	Nanochip (solid chip)	Luminex (liquid chip)	TaqMan (real time)
Pathogens covered	Flu-A, Flu-B, PIV-1, PIV-2, PIV-3, RSV	Flu-A, Flu-B, PIV-1, PIV-2, PIV-3, PIV-4, RSV-A, RSV-B, hMPV, RhV, EnV, SARS CoV	Flu-A, Flu-B, PIV-1, PIV-2, PIV-3, RSV, hMPV
No. of reactions needed	1	1	7
Hands-on time (min)[a]	70	55	135
Detection throughput (no. of tests/run)	80	96	13.5
Test turnaround time (h)[b]	9	6	4

[a] Hands-on and turnaround times include time needed for specimen processing and data analysis.
[b] Times are based on a full run, including specimen processing, target amplification, and product identification.

TABLE 3. Enhancement of clinical diagnosis by multiple testing

Virus	Test ordered			Test not ordered		
	No. of specimens tested	No. of specimens positive	% Positive specimens	No. of specimens tested	No. of specimens positive	% Positive specimens
Flu-A	197	27	13.7	163	2	1.2
Flu-B	197	10	5.1	163	0	0.0
PIV-1	93	7	7.5	267	9	3.4
PIV-3	93	8	8.6	267	10	3.7
RSV	281	29	10.3	79	1	1.3

were determined for specimens in which these viruses were not sought by the clinician.

DISCUSSION

In this study, we evaluated two multiplex RT-PCR-based in vitro RNA amplification and identification products, NGEN and ResPlex II, which allowed simultaneous detection of a panel of common respiratory viral pathogens in a single reaction. Automated microarray fluorescent probe detection provided high-throughput identification of RT-PCR amplification products. Both product assays can be performed daily with acceptable sensitivities and specificities and might be able to be used as universal platforms to develop and implement assays to detect and differentiate a panel of microbial pathogens in a timely manner.

The NGEN and ResPlex II products exhibited specificities comparable to cell culture and monoplex real-time RT-PCR for each of the six viruses detected, although a small but definite false-positive rate was observed for NGEN. Sensitivities of the two assays were lower than those of the monoplex real-time RT-PCR assays, most noticeably for RSV and PIV-3. It was noted that culture was performed on fresh specimens, whereas the molecular assays were performed on specimens which went through freeze and thaw cycles at least once. Although these might be improved by further primer/probe optimization, changes in the primer/probe sequences could have deleterious effects on other assay targets in the multiplexed reaction. Moreover, patients in our study were mostly infants or young children, who would be expected to shed virus at higher titers. It may be more of a challenge for these assays to achieve comparable sensitivity for older children and adults, who shed fewer viruses during acute infection. Nevertheless, we surmise that these two products possess an application niche in the clinical setting for rapid screening and detection of a panel of respiratory viral pathogens based on the following facts: (i) excellent specificities and favorable sensitivities in comparison to most rapid viral antigen tests; (ii) compatibility with rapid, automated, high-throughput procedures; (iii) reduced manpower, reagent, and specimen volume requirements due to simultaneous detection; and (iv) coverage of viruses for which rapid viral antigen tests are unavailable. Using molecular techniques, respiratory pathogens have been identified frequently in specimens from patients with lower respiratory tract infections (13). Furthermore, screening for a panel of respiratory viral pathogens is desirable in certain populations, such as patients receiving lung transplants, in whom levels of viral shedding in the respiratory tract are high (20, 24). This is especially desirable for the ResPlex II assay, since it covers a panel of 12 viruses.

Since many of the signs and symptoms of respiratory virus infections are similar, some respiratory virus pathogens can be overlooked when the focus is on only Flu or RSV. Our study indicates that the clinicians' diagnostic approach was highly effective in the identification of infections with Flu-A, Flu-B, and RSV, as the presence of these viruses was suspected by the ordering physician for nearly all specimens in which they were detected. In contrast, over half of PIV-1 and PIV-3 infections would have gone undetected under conditions of monoplex testing performed according to physicians' orders. Thus, multiplex testing with microarray-based detection can significantly reduce underdiagnosis of PIV infection.

Another advantage of simultaneous detection of a panel of pathogens is recognition of coinfection. When monoplex RT-PCR is used for pathogen detection, the clinician often does not consider the possible presence of other possible pathogens when given a positive result. Using the ResPlex II kit covering 12 viral pathogens, we identified nine (2.5%) specimens coinfected with two or three viral pathogens, the significance of which merits further investigation. However, a low level of cross-reactivity between PIV-1 and PIV-3 was noticed with the ResPlex II system. Coinfection with PIV-1 and PIV-3 could be missed when only detection of the virus with the higher MFI value is considered to represent a positive result.

An additional advantage of the ResPlex II system is that it also covers RhVs and EnVs. Respiratory EnVs have been demonstrated to be important causes of respiratory infections (30). In our study, ResPlex II detected 25 (6.9%) RhV infections, including 1 RhV-hMPV and 2 RhV–PIV-3 coinfections. Although RhVs are most commonly associated with mild upper respiratory tract illnesses, they have also been described in association with severe, acute lower respiratory tract infections in children, the elderly, and immunosuppressed patients. The scope, severity, and frequency of RhV-related diseases are greater when molecular methods are used for their detection (21, 28, 39). A new RhV genotype was demonstrated to cause a high incidence of influenza-like illness in New York state during 2004 and 2005 (25). These data indicate that expanded virus testing covering the most common viral pathogens as well as rhinoviruses, enteroviruses, and adenoviruses should enhance diagnostic efficiency.

Neither bacteria nor DNA viruses recognized as important causes of respiratory tract infections, such as bocavirus (2, 29), adenovirus (30), and recently reported respiratory polyomavirus (1), were included in the test panels of the NGEN or ResPlex II products evaluated. Another kit, ResPlex I, which was not evaluated here, is commercially available and covers *Mycoplasma pneumoniae*, *Legionella pneumophila*, *Chlamydia pneumoniae*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Acinetobacter baumannii*, and adenovirus types 3, 4, 7, and 21 (8).

ACKNOWLEDGMENTS

We thank Anna Raymer, Melinda Caudill, Jill White-Abell, Jan Whitehurst, Valerie Creston, Karena Blanchard, and Linda Franklin for their assistance with collection and processing of clinical specimens and John Williams for providing positive hMPV controls. We also thank Nanogen Inc. and Genaco Biomedical Products Inc. for providing their respective reagents used in this study.

REFERENCES

1. **Allander, T., K. Andreasson, S. Gupta, A. Bjerkner, G. Bogdanovic, M. A. Persson, T. Dalianis, T. Ramqvist, and B. Andersson.** 2007. Identification of a third human polyomavirus. J. Virol. **81:**4130–4136.
2. **Allander, T., M. T. Tammi, M. Eriksson, A. Bjerkner, A. Tiveljung-Lindell, and B. Andersson.** 2005. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc. Natl. Acad. Sci. USA **102:**12891–12896.
3. **Atmar, R. L., B. D. Baxter, E. A. Dominguez, and L. H. Taber.** 1996. Comparison of reverse transcription-PCR with tissue culture and other rapid diagnostic assays for detection of type A influenza virus. J. Clin. Microbiol. **34:**2604–2606.
4. **Bellau-Pujol, S., A. Vabret, L. Legrand, J. Dina, S. Gouarin, J. Petitjean-Lecherbonnier, B. Pozzetto, C. Ginevra, and F. Freymuth.** 2005. Development of three multiplex RT-PCR assays for the detection of 12 respiratory RNA viruses. J. Virol. Methods **126:**53–63.
5. **Bhat, N., J. G. Wright, K. R. Broder, E. L. Murray, M. E. Greenberg, M. J. Glover, A. M. Likos, D. L. Posey, A. Klimov, S. E. Lindstrom, A. Balish, M. J. Medina, T. R. Wallis, J. Guarner, C. D. Paddock, W. J. Shieh, S. R. Zaki, J. J. Sejvar, D. K. Shay, S. A. Harper, N. J. Cox, K. Fukuda, and T. M. Uyeki.** 2005. Influenza-associated deaths among children in the United States, 2003–2004. N. Engl. J. Med. **353:**2559–2567.
6. **Boivin, G., S. Cote, P. Dery, G. De Serres, and M. G. Bergeron.** 2004. Multiplex real-time PCR assay for detection of influenza and human respiratory syncytial viruses. J. Clin. Microbiol. **42:**45–51.
7. **Briese, T., G. Palacios, M. Kokoris, O. Jabado, Z. Liu, N. Renwick, V. Kapoor, I. Casas, F. Pozo, R. Limberger, P. Perez-Brena, J. Ju, and W. I. Lipkin.** 2005. Diagnostic system for rapid and sensitive differential detection of pathogens. Emerg. Infect. Dis. **11:**310–313.
8. **Brunstein, J., and E. Thomas.** 2006. Direct screening of clinical specimens for multiple respiratory pathogens using the Genaco respiratory panels 1 and 2. Diagn. Mol. Pathol. **15:**169–173.
9. **Coiras, M. T., P. Perez-Brena, M. L. Garcia, and I. Casas.** 2003. Simultaneous detection of influenza A, B, and C viruses, respiratory syncytial virus, and adenoviruses in clinical samples by multiplex reverse transcription nested-PCR assay. J. Med. Virol. **69:**132–144.
10. **Deng, X., H. Li, and Y. W. Tang.** 2003. Cytokine expression in respiratory syncytial virus-infected mice as measured by quantitative reverse-transcriptase PCR. J. Virol. Methods **107:**141–146.
11. **Fan, J., K. J. Henrickson, and L. L. Savatski.** 1998. Rapid simultaneous diagnosis of infections with respiratory syncytial viruses A and B, influenza viruses A and B, and human parainfluenza virus types 1, 2, and 3 by multiplex quantitative reverse transcription-polymerase chain reaction-enzyme hybridization assay (Hexaplex). Clin. Infect. Dis. **26:**1397–1402.
12. **Freymuth, F., A. Vabret, D. Cuvillon-Nimal, S. Simon, J. Dina, L. Legrand, S. Gouarin, J. Petitjean, and J. Brouard.** 2006. Comparison of multiplex PCR assays and conventional techniques for the diagnostic of respiratory virus infections in children admitted to hospital with an acute respiratory illness. J. Med. Virol. **78:**1498–1504.
13. **Garbino, J., M. W. Gerbase, W. Wunderli, C. Deffernez, Y. Thomas, T. Rochat, B. Ninet, J. Schrenzel, S. Yerly, L. Perrin, P. M. Soccal, L. Nicod, and L. Kaiser.** 2004. Lower respiratory viral illnesses: improved diagnosis by molecular methods and clinical impact. Am. J. Respir. Crit. Care Med. **170:**1197–1203.
14. **Gröndahl, B., W. Puppe, A. Hoppe, I. Kuhne, J. A. Weigl, and H. J. Schmitt.** 1999. Rapid identification of nine microorganisms causing acute respiratory tract infections by single-tube multiplex reverse transcription-PCR: feasibility study. J. Clin. Microbiol. **37:**1–7.
15. **Gruteke, P., A. S. Glas, M. Dierdorp, W. B. Vreede, J. W. Pilon, and S. M. Bruisten.** 2004. Practical implementation of a multiplex PCR for acute respiratory tract infections in children. J. Clin. Microbiol. **42:**5596–5603.
16. **Han, J., D. C. Swan, S. J. Smith, S. H. Lum, S. E. Sefers, E. R. Unger, and Y. W. Tang.** 2006. Simultaneous amplification and identification of 25 human papillomavirus types with Templex technology. J. Clin. Microbiol. **44:**4157–4162.
17. **Henrickson, K. J.** 2005. Cost-effective use of rapid diagnostic techniques in the treatment and prevention of viral respiratory infections. Pediatr. Ann. **34:**24–31.
18. **Hindiyeh, M., D. R. Hillyard, and K. C. Carroll.** 2001. Evaluation of the Prodesse Hexaplex multiplex PCR assay for direct detection of seven respiratory viruses in clinical specimens. Am. J. Clin. Pathol. **116:**218–224.
19. **Hu, A., M. Colella, P. Zhao, F. Li, J. S. Tam, R. Rappaport, and S. M. Cheng.** 2005. Development of a real-time RT-PCR assay for detection and quantitation of parainfluenza virus 3. J. Virol. Methods **130:**145–148.
20. **Jaramillo, A., F. G. Fernandez, E. Y. Kuo, E. P. Trulock, G. A. Patterson, and T. Mohanakumar.** 2005. Immune mechanisms in the pathogenesis of bronchiolitis obliterans syndrome after lung transplantation. Pediatr. Transplant. **9:**84–93.
21. **Johnston, S. L., G. Sanderson, P. K. Pattemore, S. Smith, P. G. Bardin, C. B. Bruce, P. R. Lambden, D. A. Tyrrell, and S. T. Holgate.** 1993. Use of polymerase chain reaction for diagnosis of picornavirus infection in subjects with and without respiratory symptoms. J. Clin. Microbiol. **31:**111–117.
22. **Keen-Kim, D., W. W. Grody, and C. S. Richards.** 2006. Microelectronic array system for molecular diagnostic genotyping: Nanogen NanoChip 400 and molecular biology workstation. Expert Rev. Mol. Diagn. **6:**287–294.
23. **Kehl, S. C., K. J. Henrickson, W. Hua, and J. Fan.** 2001. Evaluation of the Hexaplex assay for detection of respiratory viruses in children. J. Clin. Microbiol. **39:**1696–1701.
24. **Kumar, D., D. Erdman, S. Keshavjee, T. Peret, R. Tellier, D. Hadjiliadis, G. Johnson, M. Ayers, D. Siegal, and A. Humar.** 2005. Clinical impact of community-acquired respiratory viruses on bronchiolitis obliterans after lung transplant. Am. J. Transplant. **5:**2031–2036.
25. **Lamson, D., N. Renwick, V. Kapoor, Z. Liu, G. Palacios, J. Ju, A. Dean, K. St. George, T. Briese, and W. I. Lipkin.** 2006. MassTag polymerase-chain-reaction detection of respiratory pathogens, including a new rhinovirus genotype, that caused influenza-like illness in New York State during 2004–2005. J. Infect. Dis. **194:**1398–1402.
26. **Landry, M. L., and D. Ferguson.** 2000. SimulFluor respiratory screen for rapid detection of multiple respiratory viruses in clinical specimens by immunofluorescence staining. J. Clin. Microbiol. **38:**708–711.
27. **Liolios, L., A. Jenney, D. Spelman, T. Kotsimbos, M. Catton, and S. Wesselingh.** 2001. Comparison of a multiplex reverse transcription-PCR-enzyme hybridization assay with conventional viral culture and immunofluorescence techniques for the detection of seven viral respiratory pathogens. J. Clin. Microbiol. **39:**2779–2783.
28. **Loens, K., H. Goossens, C. de Laat, H. Foolen, P. Oudshoorn, S. Pattyn, P. Sillekens, and M. Ieven.** 2006. Detection of rhinoviruses by tissue culture and two independent amplification techniques, nucleic acid sequence-based amplification and reverse transcription-PCR, in children with acute respiratory infections during a winter season. J. Clin. Microbiol. **44:**166–171.
29. **Ma, X., R. Endo, N. Ishiguro, T. Ebihara, H. Ishiko, T. Ariga, and H. Kikuta.** 2006. Detection of human bocavirus in Japanese children with lower respiratory tract infections. J. Clin. Microbiol. **44:**1132–1134.
30. **Mackie, P. L.** 2003. The classification of viruses infecting the respiratory tract. Paediatr. Respir. Rev. **4:**84–90.
31. **Markoulatos, P., N. Siafakas, and M. Moncany.** 2002. Multiplex polymerase chain reaction: a practical approach. J. Clin. Lab. Anal. **16:**47–51.
32. **Osiowy, C.** 1998. Direct detection of respiratory syncytial virus, parainfluenza virus, and adenovirus in clinical respiratory specimens by a multiplex reverse transcription-PCR assay. J. Clin. Microbiol. **36:**3149–3154.
33. **Poehling, K. A., K. M. Edwards, G. A. Weinberg, P. Szilagyi, M. A. Staat, M. K. Iwane, C. B. Bridges, C. G. Grijalva, Y. Zhu, D. I. Bernstein, G. Herrera, D. Erdman, C. B. Hall, R. Seither, and M. R. Griffin.** 2006. The underrecognized burden of influenza in young children. N. Engl. J. Med. **355:**31–40.
34. **Puppe, W., J. A. Weigl, G. Aron, B. Grondahl, H. J. Schmitt, H. G. Niesters, and J. Groen.** 2004. Evaluation of a multiplex reverse transcriptase PCR ELISA for the detection of nine respiratory tract pathogens. J. Clin. Virol. **30:**165–174.
35. **Sefers, S. E., J. Rickmyre, A. Blackman, H. Li, K. Edwards, and Y. W. Tang.** 2006. QIAamp MinElute virus kit effectively extracts viral nucleic acids from cerebrospinal fluids and nasopharyngeal swabs. J. Clin. Virol. **35:**141–146.
36. **Syrmis, M. W., D. M. Whiley, M. Thomas, I. M. Mackay, J. Williamson, D. J. Siebert, M. D. Nissen, and T. P. Sloots.** 2004. A sensitive, specific, and cost-effective multiplex reverse transcriptase-PCR assay for the detection of seven common respiratory viruses in respiratory samples. J. Mol. Diagn. **6:**125–131.
37. **Tang, Y. W., P. J. Heimgartner, S. J. Tollefson, T. J. Berg, P. N. Rys, H. Li, T. F. Smith, D. H. Persing, and P. F. Wright.** 1999. A colorimetric microtiter plate PCR system detects respiratory syncytial virus in nasal aspirates and discriminates subtypes A and B. Diagn. Microbiol. Infect. Dis. **34:**333–337.
38. **Templeton, K. E., S. A. Scheltinga, M. F. Beersma, A. C. Kroes, and E. C. Claas.** 2004. Rapid and sensitive method using multiplex real-time PCR for diagnosis of infections by influenza a and influenza B viruses, respiratory syncytial virus, and parainfluenza viruses 1, 2, 3, and 4. J. Clin. Microbiol. **42:**1564–1569.
39. **Vuorinen, T., R. Vainionpaa, and T. Hyypia.** 2003. Five years' experience of reverse-transcriptase polymerase chain reaction in daily diagnosis of enterovirus and rhinovirus infections. Clin. Infect. Dis. **37:**452–455.
40. **Weinberg, G. A., D. D. Erdman, K. M. Edwards, C. B. Hall, F. J. Walker, M. R. Griffin, and B. Schwartz.** 2004. Superiority of reverse-transcription polymerase chain reaction to conventional viral culture in the diagnosis of acute respiratory tract infections in children. J. Infect. Dis. **189:**706–710.
41. **Woo, P. C., S. S. Chiu, W. H. Seto, and M. Peiris.** 1997. Cost-effectiveness of rapid diagnosis of viral respiratory tract infections in pediatric patients. J. Clin. Microbiol. **35:**1579–1581.